DIETARY INFLUENCES ON ADIPOSE TISSUE COMPOSITION IN THE MIGRATORY INDIGO BUNTING (*Passerina cyanea*)

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To my wife, Marcia, who waited
patiently and lovingly, for a long time,
this dissertation is dedicated

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DIETAR[illegible]SITION
IN THE [illegible] *cyanea*)

By

David Land[illegible]

December, 19[illegible]

Chairman: Dr. David W. Johnston
Major Department: Zoology

Relationships between fatty acid composition of diets and adipose tissue were investigated in captive Indigo Buntings (*Passerina cyanea*). After an 8- to 24-hour starvation period, caged birds in different groups were given four synthetically prepared diets: a high unsaturated fat diet, a high saturated fat diet, a minimal fat diet, and a "control" diet. Biopsies of the interfurcular fat pad were taken from birds on each of these diets at 2, 4, 6, 8, 10, 22 and 26 weeks after introduction of the diets. By using gas-liquid chromatography, the percent concentration of the major fatty acid components in the diets and the biopsies was determined.

The major fatty acids recorded from both the food and the biopsies were oleic, palmitic, linoleic, stearic, palmitoleic, and myristic. The

high unsaturated fat diet seemed to influence fatty acid levels in depot fat within 6 10 weeks, i e , the percent concentration of oleic acid, palmitic acid, linoleic acid, stearic acid, and palmitoleic acid in the depot fat tended to approach the levels of these acids in the diet after this period of time. The effects of the high saturated fat diet and the control fat diets on depot fat were uncertain. The minimal fat diet seemed not to influence the fatty acid levels in depot fat, i.e., oleic acid, linoleic acid, and palmitic acid levels in depot fat either were not altered or changes could not be definitely correlated with dietary levels of these fatty acids. The relationship between dietary and metabolically controlled depot fatty acids is discussed

Adipose [illegible] lized connective tissue having the capability to store [illegible] specific cytoplasmic vacuoles This tissue can be thought of as consisting of two lipid portions, a "variable" stored or "depot" fat portion that is composed mainly of triglycerides (up to 95 percent of the tissue according to Hilditch, 1964) and a phospholipid portion of the cell structure (Gunstone, 1958) The main purpose of adipose tissue is as a reserve fuel supply Upon oxidation, more calories per gram of fat are produced (9.3 kcal/gm) than for equivalent amounts of carbohydrates or protein (both of which yield about 4kcal/gm) (White, *et al*, 1964)

The depot fat portion of the adipose tissue in birds, as in mammals, can originate in two ways Depot fat can be mainly dietary in origin (exogenous), or it can be a function of intermediary metabolism (endogenous), or a combination of the two (Hilditch, 1964) Food that is not immediately oxidized may be transformed into triglycerides and stored in depot fat for use at a later time If this transformation occurs, the chemical composition of the depot fat may simply reflect the composition of the dietary fat or be a product of metabolic conversion by the animal (Shorland, 1950). Variations in chemical composition of the depot fat may be reflected in changes in the kinds of fatty acids present in the triglycerides Fatty acids may vary in chain length or

in the amount of unsaturation

Studies on the composition of depot fat in birds have emphasized as causative those factors associated with diet rather than those associated with metabolic conversion. Supporting the dietary influences on fat composition are several studies devoted to chickens, game birds, and some migratory types. Cruikshank (1934), Feigenbaum and Fisher (1959), Isaaks, *et al.* (1964), Mickelberry, *et al.* (1966), and Sell, *et al.* (1968) noted the effects of dietary fat on specific fatty acids deposited in adipose tissue of chickens. For example, chickens fed a diet containing 10 percent animal tallow showed an increased percentage of stearic acid (18:0) compared to those fed a control diet low in fat for seven weeks. Chickens fed a 10 percent soy bean oil diet (high in linoleic acid, 18:2) showed a comparable increase in depot levels of that fatty acid (Mickelberry, *et al.*, 1966).

Four non-migratory tetraonids were studied by Moss and Lough (1968). In the Red Grouse (*Lagopus lagopus scoticus*), Ptarmigan (*Lagopus mutus*), Black Grouse (*Lyrurus tetrix*), and Capercaillie (*Tetrao urogallus*) collected in northeast Scotland, fatty acid analyses of depot fat showed a high proportion of linoleic acid (18:2) and linolenic acid (18:3). These two acids were also found to be proportionately high in the diets of both the Red Grouse and Ptarmigan. (They do not have dietary data for the other two birds.) Linoleic acid (18:2) represented 29-34 percent of the total fatty acids present in the Red Grouse's depot fat. The bird's main food supply, heather (*Calluna vulgaris*), contained approximately

20 percent of this [illegible]

Tanhaunpaa and [illegible] found that the fatty [illegible] composition in the [illegible] matched the fatty acid composition of [illegible] on the similarity between linoleic [illegible] in the birds and the similar values in their food supply (*Vaccinium* and *Empetrum* spp.).

Similarly in the Willow Ptarmigan (*Lagopus lagopus*) dietary fat seems to dictate the fatty acid composition of the depot fat (West and Meng, 1968a). Willow twigs and buds, comprising 97 percent of the ptarmigan's diet, change in fatty acid composition seasonally. Corresponding changes were found in certain fatty acids from interclavicular adipose tissue taken from the birds in corresponding seasons.

Other studies have dealt with migratory species in which influences of dietary fat composition on the adipose tissue composition have been suspected. McGreal and Farner (1956) measured saponification number (a measure of fatty acid chain length) and iodine number (a measure of the degree of unsaturation) in vernal premigratory fat of the Gambel's White-crowned Sparrow (*Zonotrichia leucophrys gambelii*). No changes in either measure were detected when the thoracic fat pads were analyzed during the premigratory period (18 February to 30 April). In the skin and carcass, however, slight changes in the saponification number were noted during this period. McGreal and Farner suggested that such an alteration could be dietary in origin.

Nakamura (1952, 1963) also measured saponification number in body lipids of the migratory Eastern Great Reed Warbler (*Acrocephalus arundi-*

naeus orientalis). An increase in saponification number (suggesting a decrease in mean chain length) was reported for the premigratory and migratory periods No mention was made of any dietary influences on the observed changes in saponification number In determining iodine numbers, however, a similarity between stomach contents and total body lipid extracts was reported

Analyses of the fatty acid composition of migratory birds were initiated by Walker (1964). She studied the percent composition of the six major fatty acids from fat body extracts in three insectivorous birds, Magnolia Warbler (*Dendroica magnolia*), Tennessee Warbler (*Vermivora peregrina*), and Red-eyed Vireo (*Vireo olivaceus*), and the granivorous Bobolink (*Dolichonyx oryzivorous*) Greater similarities were found among the insectivores when compared to each other than when compared to the granivore As a minor point, Walker commented " .it is possible that the nature of the food ingested had some effect on the composition of the fat." Walker did not, however, attempt to discern the fatty acid composition of the specific foods of each of these species.

Bower and Helms (1968) investigated fatty acid composition in the migratory Slate-colored Junco (*Junco hyemalis*) In extracting fatty acids from the whole carcass they noted certain seasonal variations For example, winter levels of linoleic acid (18 2) were proportionately high but decreased in the spring, whereas oleic acid (18 1) and palmitic acid (16 0) levels varied conversely These changes were believed to be correlated with a seasonal dietary change--the Junco switches from a seed diet rich

in linoleic acid in winter to an insectivorous diet relatively higher in oleic acid and lower in linoleic acid in the spring (Hilditch, 1964). Bower and Helms concluded therefore that the change in depot fat was probably due to diet.

Cited above are studies wherein the authors have hypothesized that some birds may change their diet and thereby alter the nature of their depot fat (Walker, 1964, Bower and Helms, 1969). Several field reports substantiate these laboratory hypotheses. Dolnik and Blyumental (1967) found that Chaffinches (*Fringilla coelebs coelebs*) change from eating insects in the summer to seeds in the fall. Yarbrough and Johnston (1965) noted a seasonal change from fruits to insects in the Myrtle Warbler (*Dendroica coronata*). The Black-faced Dioch (*Quelea quelea*) of Nigeria has a short period of high intensity termite-feeding which occurs prior to a 200 Km. southern flight in the rainy season (Ward, 1965). Fry (1967) observed a change in diet in the Bee-eater, *Merops albicollis*, in its winter quarters. He stated, " ..the premigratory accumulation of fat in *M. albicollis* is associated with the change in composition of the diet."

Papers suggesting an endogenous control of the chemical composition of depot fat in birds are few. West and Meng (1969b) compared the fatty acid composition of the diet and the interclavicular fat pad of the migratory Redpoll (*Acanthis flammea*). In addition to the comparison between diet and depot fat, they also compared depot fats of feral and captive birds. Some birds were kept in captivity and maintained on lab diets for six weeks prior to analysis. Fatty acid composition of the depots were

found to be similar to each other regardless of the fatty acid composition of the three diets. The fatty acid composition of the wild bird's fat was different from that of both the captive birds and their principal natural food (*Chenopodium* seed). West and Meng concluded that the "physiological state of the bird (migration, breeding, etc.) exerts a greater effect on the fatty acid composition of the bird's depot fat than does diet *per se*."

In an analysis of the fatty acid composition of depot fat, heart and muscle of the Wood Thrush (*Hylocichla mustelina*) Rosen (1966) raported a decrease in saturated fatty acids in these tissues during the breeding season. Linolenic acid (18:3) increased during the same period whereas the essential fatty acids, linoleic (18:2) and arachidonic (20:4), did not change their levels. (Arachidonic (20:4) was present in the muscle and liver but was absent in depot fat.) In addition to linolenic acid levels changing during the summer, Rosen also noted a decrease in the linoleic-palmitic acid ratios (18:2/16:0) in the three tissues analyzed when summer ratios were compared to fall premigratory values. While Rosen pointed out that the food eaten during the summer months and subsequent fall pre-migratory period might change, he felt that the changes in tissue fatty acid composition were probably under metabolic rather than dietary influence.

Barnett (1970) discussed seasonal changes in fatty acids of total body extracts from the House Sparrow (*Passer domesticus*). He found that iodine numbers, oleic acid (18:1) and docosahexanoic acid (22:6) levels were greater in the winter than in the summer while stearic acid (18:0) and linoleic acid (18:2) levels decreased from summer to winter. He implies that the changes in iodine number and fatty acid percent composition when

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In order to [illegible] due to exogen[illegible] during periods [illegible] occur. In many migratory birds, a rapid and marked increase in fat deposition occurs during the premigratory and migratory period. In the spring, for example, significant increases in body weight in the form of fat reserves have been reported (Odum and Major, 1956, Odum, 1960, Odum and Perkinson, 1961). These increases in fat deposition may be accompanied by changes in diet (Rower and Helms, *op. cit.*). If feral migratory birds alter their diets during periods of fat deposition, they might be increasing specific fatty acid levels or simply changing to whatever diet is available. In both cases, tissue fatty acid levels would probably be equivalent to those in the diet. If they do not change their diet during periods of increased lipid deposition, they may maintain already existing diet-dependent tissue fatty acid levels or they may metabolically alter certain fatty acid synthesizing pathways. In the latter case, tissue level fatty acids could be dissimilar to dietary fatty acid levels.

In order to determine influences of specific dietary fatty acids on fat depots in a migratory bird, captive Indigo Buntings (*Passerina cyanea*) were placed on four special diets at Gainesville, Florida in 1969. In each, the fatty acid composition was different. While birds were on these diets, biopsies were taken of the interfurcular fat pad over a 5 1/2-month period from April to October. Fatty acid composition from depot fats

taken during these periods was compared to their respective diets The goals of the study were to

(1) determine, at intervals, the fatty acid composition of caged Indigo Buntings' adipose tissue,

(2) determine the effects of high unsaturated, high saturated and low fat diets on the fatty acid composition of the adipose tissue over a period of time,

(3) help clarify the degree of influence dietary fatty acids might have on lipid deposition, in this migratory species

METHODS

Trapping and Caging Methods

The Indigo Buntings obtained for this study were caught in mist nets in the spring and fall of 1968. The 16 captured in the spring (March-April), were brought in from Homestead, Florida, to Gainesville in May, 1968, whereas the remaining 15 were taken in a grassland habitat on the University of Florida campus during the period from September 29 to October 24. All birds were sexed and aged by using color and other differences previously assessed by Johnston (1965).

In November the birds were divided into four groups as follows: Group 1 contained 5 males and 3 females, Groups 2 and 3 each contained 4 males and 4 females, Group 4 contained 5 males and 2 females. All the indoor cages were 3 ft. x 6 ft. x 6 ft. and located directly in front of closed windows. The room in which the birds were kept was at constant temperature (ca. 25°C) and subjected to natural photoperiods. Birds were weighed in the morning at 7 14-day intervals from October, 1968, to April, 1970, to record any seasonal fluctuations in weight comparable to those reported for feral buntings (Johnston, 1965).

Preparation and Composition of Diet

All birds were placed on a diet of fine-grain chick feed from the

beginning of November, 1968, through March, 1969, a period of five months. Water was supplied *ad libitum*. On March 31, all food was removed for 8-48 hours in order to reduce the magnitude of existing fat depots. After this starvation period, the four groups were each subjected to a different experimental diet, the compositions of which are given in Table 1. The diets were named according to their relative abundance of fat and whether they were high in unsaturated or saturated fatty acids. Birds in Group 1 were subjected to a diet consisting of 14.9 percent fat. This diet was high in unsaturated fatty acids because safflower oil was used to increase the total fat percentage. Birds in Group 2 were subjected to a diet consisting of 12.9 percent fat. This diet was high in saturated fatty acids because animal tallow was used to increase the total fat percentage. Birds in Group 3 were subjected to a diet with less than 1 percent total fat and birds in Group 4 were subjected to a diet of 4.9 percent fat. The 4.9 percent fat in the diet given to Group 4 was found to be equivalent to the percent of fat in the fine-grain chick feed which the birds were eating prior to the starvation period. This latter diet was therefore considered a laboratory control.

Method of Collecting and Preparing samples for Gas-Liquid Chromatography

Adipose tissue biopsies were taken from the interfurcular fat pad of unanesthetized birds at specified intervals. From 1-10 mg. of fat was removed, two sutures were sufficient to insure healing. The first biopsies were taken from 27 of the 31 birds on March 29, 1969, prior to the starvation period and introduction of experimental diets. (Four birds

TABLE 1

COMPOSITION OF FOUR EXPERIMENTAL DIETS[1]

Ingredients	1 High Unsaturated Fat	2 High Saturated Fat	3 Minimal Fat	4 Control Fat
Corn	51.8 g/100g	51.8 g/100g	-	63.3 g/100g
Degerminated corn	-	-	63.8 g/100g	-
Soybean Meal (50% protein)	29.3	29.3	27.8	27.8
Fish Meal (60% protein)	3.0	3.0	3.0	3.0
Alfalfa Meal (20% protein)	3.0	3.0	3.0	3.0
Ground Limestone	0.6	0.6	0.6	0.2
Defluorinated PO_4 (18% P-32% Ca)	1.4	1.4	1.3	1.8
Iodized Salt	0.4	0.4	0.4	0.4
Animal Tallow	-	10.0	-	-
Safflower Oil	10.0	-	-	-
Vitamin Premix[2]	0.4	0.4	0.4	0.4
Percentage protein	22.0	22.0	22.0	22.0
Productive energy (cal /Kg	2388	2388	1079	1079
Percentage fat (Chloroform methanol extraction)	14.9	12.9	0.9	4.9

1. these diets were prepared with the help and guidance of Dr. Robert Harms, Poultry Science Department, University of Florida
2. Standard supplements of essential vitamins and minerals.

did not have a large enough fat pad to permit biopsy) Five successive samples were taken at two-week intervals: April 16-17, May 3 4, May 17-18, May 31-June 1, and June 14 Two more samples on September 20 and October 11 were also included to observe any long-term effects.

Whereas 27 samples were collected at the first biopsy, March 29, during the next three months, the captive birds irregularly lost weight to such an extent that an interfurcular fat pad biopsy could not be carried out on all birds due to diminution in size throughout this period. Thus, although the study initially included 31 birds, collecting samples for the first seven of the eight periods was successful on only four birds, one on each diet In addition to the four profiles representing each diet, five biopsies were collected at one time from one bird that died subsequently during the second sampling period. These latter samples were taken to assess any variability of the fatty acid composition from one region of the interfurcular fat pad to the next, and were randomly chosen from different sections of the fat pad at various depths

Gas-liquid Chromatography Procedure

Biopsies were weighed and stored frozen until further analysis. Each sample was ultimately homogenized in chloroform-methanol (2:1), double filtered, and the extract collected in a refluxing vessel (Folch, et al., 1951). After removal of chloroform-methanol by flash evaporation (at %)°C.) the sample was saponified with 20 ml. of 10 percent methanolic KOH for 12-24 hours Methanol was then removed with the flash evaporator

and the saponified fraction washed into a separatory funnel with distilled water. The fatty acid salts were separated from the non-saponifiable fraction by washing four times with petroleum ether (b.p. 30°-60°C.) after which the aqueous phase was saved. The salts were then converted to their respective acids by acidifying with 5N H_2SO_4. Three subsequent washings with petroleum ether were carried out to collect the fatty acids. The petroleum ether phase was evaporated as before and the residue quantitatively transferred to a test tube by three 1 ml. washings with reagent-grade hexane. Methylation was accomplished by adding 0.5 ml. BCl_3-methanol reagent to the hexane fraction and placing the sample over a steam bath for five minutes (Hames, 1967).

Qualitative and relative quantifications of the methyl esters of the component fatty acids from each sample were made with a Varian Aerograph, Model 600-D, gas chromatograph containing a flame ionization detector operated isothermally at 198°C. The column used was stainless steel (1/8" x 6') packed with 10 percent DEGS (diethyleneglycol succinate) as the stationary phase on 60/80 chromosorb P. Oven temperature was 198°C. and the flow rates were N_2=32 ml./min. and H_2=28 ml./min. Standard methyl esters for comparing relative retention times were obtained from Applied Science Laboratories, State College, Pennsylvania. Percentages of each fatty acid were calculated by measuring height and relative retention time (Horning, *et al.*, 1964).

RESULTS

Body Weights of Caged Indigo Buntings

Body weights for the captive Indigo Buntings over a 16-month period are presented in Figure 1. The graph depicts two general mid winter weight peaks evidently due to increased fat depots. The first, in December of 1968, indicates a mean weight for 31 birds of 18.4 gm. The second, in December, 1969, is higher, at a mean weight of 20.8 gm. for 19 birds. The lowest natural mean weight occurred in September, 1969; 15.5 gm. for 20 birds. An artificial "low" weight was produced after the 8 - 48-hour starvation period at the end of March, 1969. The mean weight for 25 birds after starvation was 14.4 gm. Although variations in mean body weight are apparent, an analysis of repeated measures (Morrison, 1967) showed that the highest and lowest mean weights were not significantly different statistically ($F=1.90$, df 12,8) with the exception of the artificially induced starvation weight. The mean body weight after the 8 - 48-hours of starvation was significantly different when compared to the mean body weight two days before it ($F=13.5$, df 2, 25, $p < .01$). The mean body weight after starvation was also significantly different when compared to the mean body weight two weeks later ($F=45.5$, df 2,25, $p < .001$). Observations of the interfurcular fat pad during the starvation period tended to support the view that reduction in body weight was accompanied by a reduction in fat pad size. A noticeable increase in fat pad size

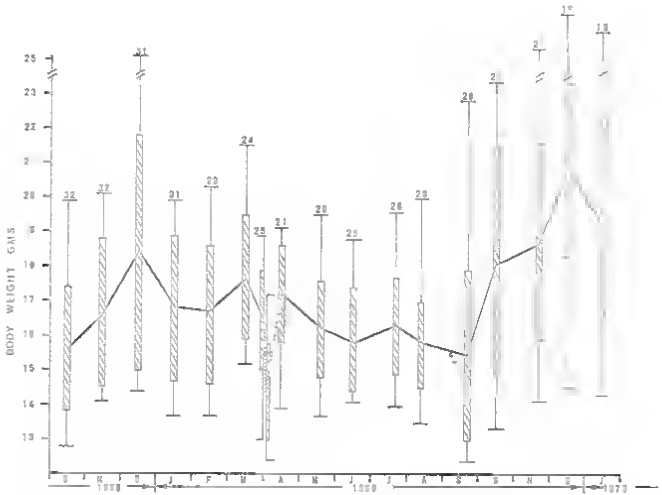

Figure 1. Body weights of captive Indigo Buntings Vertical lines indicate the range, the rectangle is ± one standard deviation and the number at the top of each range is the sample size

correl[illegible] two weeks on the experimental [illegible]

Analysis of F[illegible]

Biopsies from p[illegible]

The first biopsy was taken from 27 of the 31 birds on March 24, prior to the starvation period. Analysis of the fatty acid composition of a sample from the interfurcular fat pad of each bird is presented as a percentage of the total fatty acid complement (Table 2). The results of a univariate analysis of variance indicated that the percentage of each fatty acid was not significantly different between groups. The results of a multivariate analysis of variance indicated that the profile of the six major fatty acids, myristic (14 0), palmitic acid (16 0), palmitoleic acid (16 1), stearic acid (18 0), oleic acid (18 1), and linoleic acid (18 2), together, showed no significant differences between each group.

Synthetic experimental diets

The percentage of fat content per diet is presented in Table 3. This percentage was determined by chloroform-methanol extraction performed on a sample of each diet. The results from two samples of each diet were averaged. This table also contains the percentages of each fatty acid in each diet. In the high unsaturated fat diet, 63 percent of the total fatty acids present was linoleic acid and the total of unsaturates in this diet was 83 percent. The high saturated fat diet based on animal tallow con-

PERCENT [illegible] FAT ADIPOSE TISSUE BIOPSIES

Fatty Acid	GROUPS 1 N-6 Mean ± S.D.	2 N-7 Mean ± S.D.	3 N-7 Mean ± S.D.	4 N-7 Mean ± S.D.	All Groups N-7 Mean ± S.D.
14.0	2.46 ± 2.12	3.11 ± 1.46	2.23 ± 0.69	3.23 ± 0.38	2.83 ± 1.37
16.0	26.34 ± 5.26	30.61 ± 5.05	32.40 ± 3.10	28.35 ± 5.32	29.36 ± 5.19
16.1	6.45 ± 2.08	5.10 ± 1.65	6.20 ± 1.94	5.84 ± 1.58	6.10 ± 1.80
18.0	6.38 ± 1.89	7.73 ± 1.74	5.16 ± 2.03	5.15 ± 1.49	6.08 ± 1.77
18.1	42.72 ± 3.74	40.13 ± 5.95	39.91 ± 2.80	40.15 ± 3.61	40.66 ± 4.29
18.2	14.84 ± 6.53	11.74 ± 2.80	14.12 ± 4.14	15.60 ± 4.44	14.43 ± 4.57

TABLE 3

PERCENTAGE FAT AND FATTY ACID COMPOSITION OF SYNTHETIC DIETS

	High Unsaturated Fat Diet	High Saturated Fat Diet	Minimal Fat Diet	Control Fat Diet
Percent fat per diet	14.9	12.9	0.9	4.9
14:0 per total fatty acids	0.23	1.68	1.20	4.89
16:0 per total fatty acids	13.00	24.35	21.84	37.00
16:1 per total fatty acids	0.40	2.41	3.55	7.43
18:0 per total fatty acids	3.55	18.40	5.20	15.14
18:1 per total fatty acids	20.01	36.20	21.29	28.95
18:2 per total fatty acids	62.78	16.80	42.61	6.56

turned 44 per[illegible] fatty acids as saturated fatty acids. The minimal fat [illegible] t 28 percent of its fatty acid components as saturated [illegible] control diet (i.e., where the fat portion constitute[illegible] of the total food complement) 57 percent of the total fatty acid complement was saturated.

Variability of fatty acid composition in an interfurcular fat pad

The analysis of the fatty acid composition of five samples taken simultaneously from different regions of the same fat pad is presented in Table 4. The bird being sampled, died when the second biopsy was taken, making it feasible to take several samples from the same fat pad in the same bird. Little variation is seen in percentages of the major components (linoleic, oleic and palmitic acids). Variation in the minor components (myristic, palmitoleic and stearic) can be compared to the means and standard deviations of these fatty acids in the pre-diet biopsy (Table 2). By taking $\pm$ 2 standard deviations from the means of these three fatty acids, the variability observed in Table 4 is not significant. Because linoleic acid (18 3) did not occur in the pre-diet biopsies, a comparison between the samples from the same bird and the pre-diet biopsies cannot be made and the variability between linolenic acid levels cannot be tested at this time.

Depot fatty acid composition in birds on four experimental diets

The profiles of the fatty acid composition of four birds are presented in Table 5. The table indicates, first, the percent fatty acid composition

TABLE 4

FATTY ACID PERCENT COMPOSITION OF FIVE BIOPSIES
TAKEN AT ONE TIME FROM ONE BIRD

Fatty Acid	1	2	3	4	5
14:0	1.98	1.54	1.84	0.44	0.65
16:0	20.37	27.20	25.12	19.11	19.42
16:1	3.80	2.64	2.29	1.31	2.91
18:0	3.30	5.60	6.30	7.08	7.52
18:1	24.00	22.83	23.61	29.07	26.53
18:2	43.77	36.06	37.43	42.33	41.54
18:3	2.24	4.12	3.38	0.62	1.38

TABLE 6

PERCENT FATTY ACID COMPOSITION OF
ADIPOSE TISSUE BIOPSIES OF FOUR DIETS

Fatty Acid	Percent Fatty Acid in Diet	Biopsy							
		1	2	3	4	5	6	7	8
		Weeks on Experimental Diet							
		0	8	4	6	8	10	22	26
Diet on High Unsaturated Fat Diet									
14:0	0.23	2.37	1.86	-	1.86		1.45	1.68	
16:0	15.00	26.85	31.88	19.99	25.52	16.87	15.68	20.22	
16:1	0.40	5.56	3.30	4.79	2.36	4.59	1.59	3.85	
18:0	3.65	7.95	11.53	13.86	6.84	3.71	3.01	6.10	
18:1	28.01	42.91	28.83	41.61	16.63	20.87	17.44	20.44	
18:2	52.70	14.26	12.93	20.64	47.38	55.07	60.85	41.80	
Diet on High Saturated Fat Diet									
14:0	1.68	2.52	2.30	2.81	2.44	1.22	3.50	1.10	3.27
16:0	24.35	30.87	34.37	26.26	23.58	30.45	42.11	37.61	33.46
16:1	2.41	3.90	4.23	3.55	3.00	3.78	13.64	3.13	6.66
18:0	10.46	7.49	12.52	10.76	12.41	9.46	5.84	10.61	4.84
18:1	36.20	41.55	33.33	37.82	45.76	43.86	27.78	43.33	48.33
18:2	16.68	13.08	13.11	18.82	8.70	11.13	7.09	4.11	7.49
Diet on Minimal Fat Diet									
14:0	1.20	2.40	1.75	1.74	0.18	1.68	1.69	2.02	2.76
16:0	21.84	27.10	28.23	33.71	35.98	39.15	34.53	35.82	36.63
16:1	3.56	3.41	7.70	6.09	8.93	5.72	7.29	7.35	8.96
18:0	5.20	7.76	4.21	7.94	3.41	7.76	5.48	8.16	6.74
18:1	21.29	40.91	57.57	47.68	54.58	47.75	45.88	46.55	45.87
18:2	42.61	15.47	5.33	2.80	3.94	7.36	4.73	-	
Diet on Control Fat Diet									
14:0	4.09	2.80	0.86	3.20	5.45		1.09	4.42	
16:0	37.80	31.55	35.34	45.55	30.85	25.69	41.21	33.23	
16:1	7.43	4.84	5.02	6.53	4.16	6.80	3.53	10.03	
18:0	15.14	7.34	4.45	6.35	16.66	2.20	7.58	2.16	
18:1	28.95	43.22	48.10	36.11	35.99	30.34	37.46	38.42	
18:2	6.66	9.76	6.31	3.23	2.81	36.94	8.27	10.92	

for each diet followed by the percent composition of each biopsy. Seven biopsies were taken from the birds on the high unsaturated fat diet and the control fat diet and eight biopsies were taken from the birds on the high saturated fat diet and minimal fat diet. The information in this table can be examined in two graphic ways. In Figures 2 through 7, the effects of the four diets on each of the major fatty acids are presented. By grouping the information this way, these figures allow for the comparison of the effects of each dietary fatty acid on the same fatty acid in the adipose tissue.

Each figure, representing a single fatty acid, consists of four graphs. The graphs indicate the percentage of that fatty acid from the biopsy of a bird on one of the experimental diets. The number of weeks that the bird has been kept on its particular experimental diet is given along with the number of biopsy. Two other kinds of information are also included on each graph: the level of the fatty acid in the experimental diet and a $\pm$ 2 standard deviation range of variability. This range is based on the standard deviation determined for each fatty acid from the pre-diet biopsy (Table 2). The standard deviation range is discussed later.

The percent composition of myristic acid in the birds kept on the four diets is shown in Figure 2. The $\pm$ 2 standard deviation range of variability for this fatty acid was 0.2 percent to 5.5 percent. The levels of myristic acid in the birds on the unsaturated, saturated and minimal fat diets were within the range of variability for all biopsies. The bird on the control fat diet showed greater variability of myristic

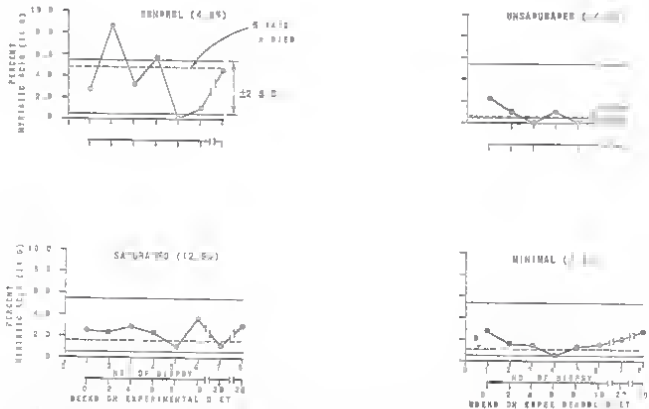

Figure 2. Percent myristic acid (14:0) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of myristic acid in a biopsy of the interfurcular fat pad of a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included

acid levels were , in biopsy number 2, myristic acid level was 8 9 percent and in biopsy number 5, myristic acid level was zero The mean levels of myristic acid in the four diets were 0 2 percent on the unsaturated fat diet, 1.7 percent in the saturated fat diet, 1 2 percent in the minimal fat diet, and 4 9 percent in the control fat diet

The percent composition of palmitic acid in the birds kept on the four diets is shown in Figure 3 The range of variability for this fatty acid was 19 0 percent to 39 8 percent. In the bird on the unsaturated fat diet, palmitic acid in biopsies 1, 2, 3, and 4 were within the range of variability. Levels of palmitic acid in biopsies 5 and 6 were below it, at 15.5 percent and 16.5 percent, respectively. In the last biopsy, the palmitic acid level rose again entering the range of variability In the bird on the saturated, minimal and control fat diets, the palmitic acid levels varied greatly while staying in or near the range of variability Mean palmitic acid levels in the unsaturated fat diet was 13 0 percent, in the saturated fat diet was 26.4 percent, in the minimal fat diet was 21 8 percent, and in the control fat diet was 37 0 percent

The percent composition of stearic acid in the birds kept on the four diets is shown in Figure 4 The range of variability for this fatty acid was 2.5 percent to 9.6 percent In the bird on the unsaturated fat diet, levels of stearic acid increased above the range of variability for biopsies 2 and 3, rising to 11 5 percent and 13.0 percent, respectively The stearic acid levels in the remainder of the biopsies from the bird on the unsaturated fat diet were between 3 0 percent and 6.1 percent, i.e.,

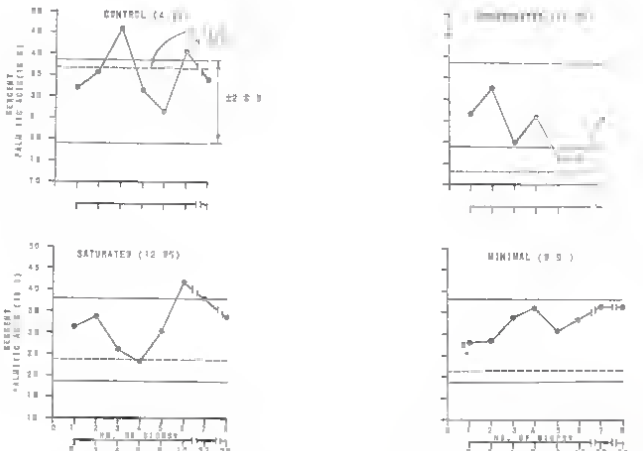

Figure 3. Percent palmitic acid (16:0) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of palmitic acid in a biopsy of the interfurcular fat pad of a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included.

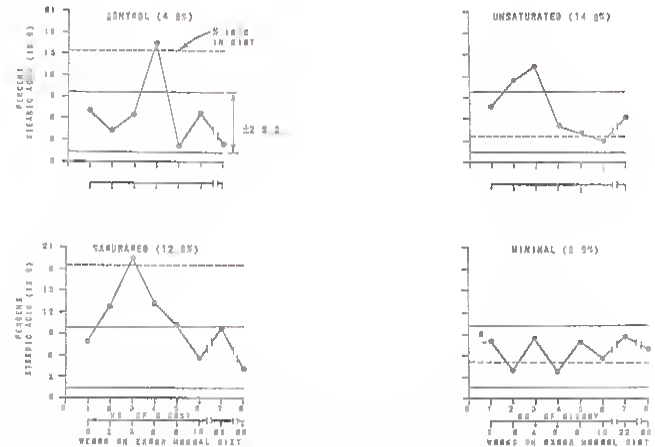

Figure 4 Percent stearic acid (18:0) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of stearic acid in a biopsy of the interfurcular fat pad of a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included.

within the range of variability for this fatty acid In the bird on the saturated fat diet, biopsies 2,3, and 4 were above the range of variability at 12 5 percent, 18 7 percent, and 12 4 percent, respectively, whereas the stearic acid levels in biopsies 5, 6, 7, and 8 of this bird were within or very close to the range of variability Stearic acid levels in the bird on the minimal fat diet were all within the range of variability for the eight biopsies Variability of stearic acid levels in the bird on the control fat diet remained in or near the range of variability for all but one biopsy Stearic acid level in biopsy 4 of this bird was 16 7 percent The mean levels of stearic acid in the four diets were: 3.6 percent in the unsaturated fat diet, 18 5 percent in the saturated fat diet, 5 2 percent in the minimal fat diet and 15 1 percent in the control fat diet

The percent composition of palmitoleic acid in the birds kept on the four diets is shown in Figure 5. The range of variability for this fatty acid was the same as that of stearic acid, i.e., 2.5 percentto 9.6 percent. In the bird on the unsaturated fat diet, palmitoleic acid levels remained within the range of variability for six of seven biopsies Biopsy 6, at 1.6 percent, was below the range of variability. On this graph we can see what appears to be a gradual reduction of the adipose tissue level of palmitoleic acid, with respect to time. This trend approaches the lower limit of the ranges of variability Palmitoleic acid levels in the birds on the other three diets remained within the range of variability for almost all biopsies, with the following exceptions. Biopsy 6, from the bird on the saturated fat diet, indicated a 13 6 percent palmitoleic acid

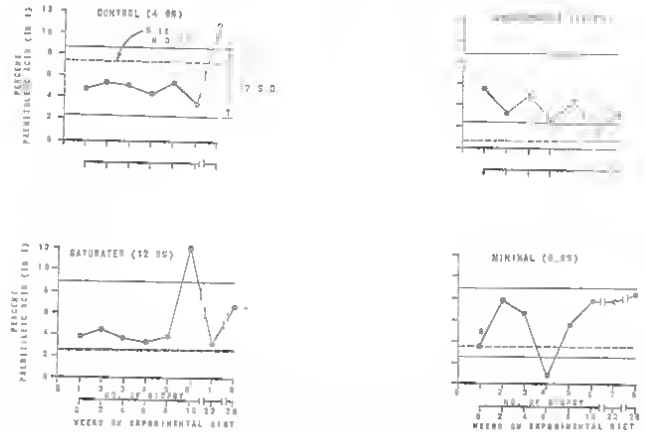

Figure 5. Percent palmitoleic acid (16:1) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of palmitoleic acid in a biopsy of the interfurcular fat pad of a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included.

level, above the r[illegible], from the bird on the minimal fat diet, d[illegible] to 0.9 percent, below the range of v[illegible] No trends in palmitoleic acids levels were apparent in the bi[illegible] the saturated, minimal or control fat diets. The mean levels of palmitoleic acid in the four diets were 0.4 percent in the unsaturated fat diet, 2.4 percent in the saturated fat diet, 3.6 percent in the minimal fat diet, and 7.4 percent in the control fat diet

The percent composition of oleic acid in the birds kept on the four diets is shown in Figure 6 The range of variability for this fatty acid was 32 1 percent to 49 3 percent Oleic acid levels in the bird on the unsaturated fat diet is seen to drop out of the range of variability in biopsy 2, down to 28 9 percent In biopsies 4, 5, 6, and 7, oleic acid levels in this bird remain at lavels between 16.6 percent and 20.8 percent Oleic acid levels in the bird on the saturated fat diet fluctuate greatly dropping below and returning to the range of variability (the low point occurred in biopsy 6, when oleic acid was 27 8 percent) Oleic acid levels in the bird on the minimal fat diet remained at or near the range of variability Oleic acid levels in the bird on the control fat diet showed a continual decline between biopsies 1 and 5, dropping to 30 3 percent, but in biopsies 6 and 7, the oleic acid increased back to the range of variability The mean levels of oleic acid in the four diets were 20.0 percent in the unsaturated fat diet, 36 3 percent in the saturated fat diet, 21 3 percent in the minimal fat diet, and 29.0 percent in the control fat diet

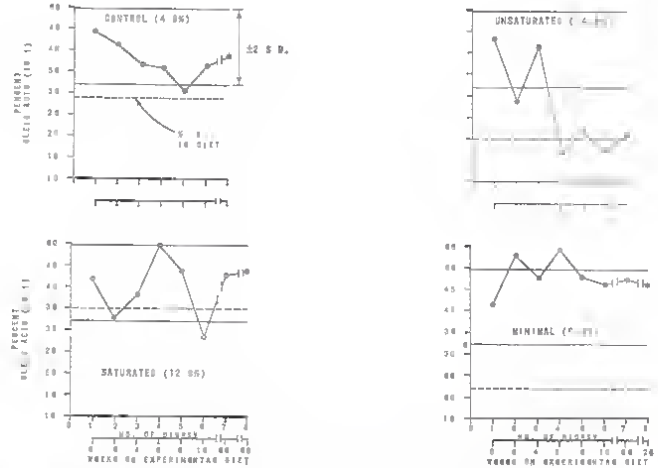

Figure 6. Percent oleic acid (18:1) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of oleic acid in a biopsy of the interfurcular fat pad in a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included.

The percent composition of linoleic acid in the birds kept on the four diets is shown in Figure 7. The range of variability for this fatty acid was 5.2 percent to 3.6 percent. Linoleic acid levels in the bird on the unsaturated fat diet continually increased from biopsy 2, at 12.9 percent to 60.9 percent in biopsy 6. A drop in biopsy 7 linoleic acid level to 41.8 percent followed. Linoleic acid levels in the saturated fat diet varied slightly but remained within the range of variability for all biopsies except biopsy 7. At a 4.1 percent linoleic acid level, it was slightly below the lower limit of the range of variability. Linoleic acid level in the bird on the minimal fat diet dropped to 2.8 percent, below the range of variability, in biopsy 3. Biopsies 4 and 5 showed increases to 7.4 percent which is within the range of variability but, again, in biopsies 6, 7, and 8, linoleic acid levels declined. Linoleic acid level in biopsy 6 was 4.7 percent, followed by zero levels in the last two biopsies. In the bird on the control diet, the linoleic acid level remained relatively constant near the lower limit of the range of variability for all biopsies except biopsy 5. In this case, the linoleic acid level rose to 35.9 percent.

In the preceding figures, the dietary fatty acid levels were compared with adipose tissue fatty acid levels. The figures presented fatty acid levels in both diet and tissue for each separate fatty acid. The data in Table 5 can be regrouped so that all tissue fatty acid percentages are presented together for each separate diet (Figures 8-11). In this way, it can be seen which fatty acids make up the major portion of each adipose tissue biopsy. Also, in these graphs, corresponding changes in the per-

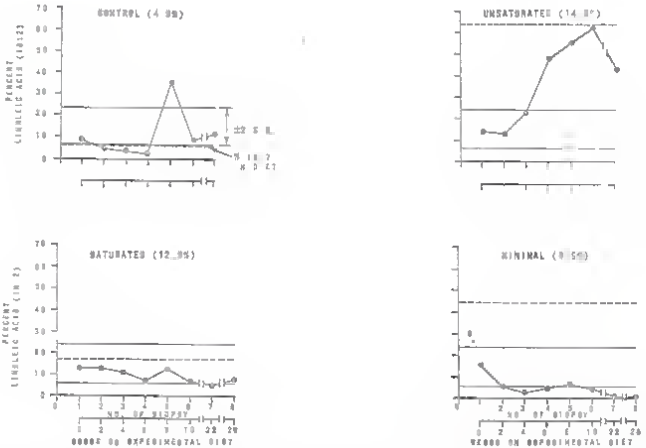

Figure 7. Percent linoleic acid (18:2) in birds on each of the four synthetic diets. Each of the four graphs represents the percent of linoleic acid in a biopsy of the interfurcular fat pad of a captive Indigo Bunting. A ± two standard deviation range of variability based on the first biopsy is also included.

centage of any o[illegible] in the [illegible] fatty acid can be detected.

The percentages of the fatty acids in the bird kept on the unsaturated fat diet are presented in Figure 8. The major components in biopsies 1, 2, and 3 were oleic and palmitic acid. Added together they constituted 60.7 percent to 69.7 percent of the total fatty acids present in these three biopsies. In the remaining biopsies they represented less than 50.0 percent of the total fatty acids present because linoleic acid (which had been less than 21.0 percent of the total fatty acids in the first three biopsies) became the dominant fatty acid. Linoleic acid represented over 40 percent of the total fatty acids present in biopsies 4, 5, 6, and 7. An interesting relationship between the present composition of palmitic and oleic acid is apparent in this figure. In biopsy 2, oleic acid level dropped, relative to its level in biopsy 1, from 42.9 percent to 28.8 percent whereas the palmitic acid level increased from 26.9 percent in biopsy 1 to 3.19 percent in biopsy 2. In biopsy 3, oleic acid increased to 41.6 percent while palmitic acid dropped to 20.0 percent. Thus, as the oleic acid level dropped, the palmitic acid level rose when biopsies 1, 2, and 3 were compared. A similar complementary relationship is seen between these two fatty acids when biopsy 4 levels are compared with biopsy 3 levels, and biopsy 5 levels are compared with biopsy 4 levels. The changes in level between biopsy 6 and 5 are not great enough for the complementary relationship to be seen and is absent when biopsy 7 is compared to biopsy 6, since the level of both fatty acids rose in the last biopsy. The levels of stearic, palmitoleic, and

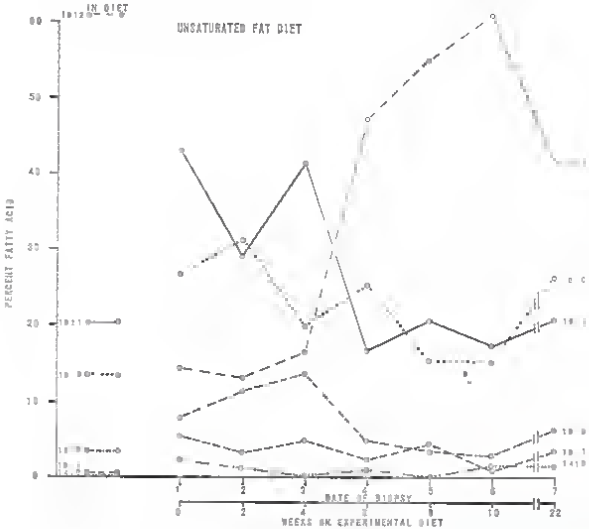

Figure 8. Effect of an unsaturated fat diet on the percent of fatty acids in an adipose tissue biopsy from the interfurcular fat pad of an Indigo Bunting.

and myristic acids constitute relatively constant and minor components of the total fatty acids in the bird kept on the unsaturated fat diet A slightly decreasing trend in stearic and palmitoleic acid may be seen from biopsies 1 through 7

The percentage of fatty acids in the bird kept on the saturated fat diet is presented in Figure 9. Oleic and palmitic acids are the major fatty acids constituting over 64 percent of the total fatty acids of every biopsy. The complementary relationship between oleic and palmitic acid levels described in the bird on the unsaturated diet is also seen here. This relationship is noted when the levels of oleic and palmitic acids from each biopsy are compared with their respective levels in the previous biopsy. For example, oleic acid is 41.6 percent of the total fatty acids present and palmitic acid is 31 percent of the total fatty acids in biopsy 1. In biopsy 2, oleic acid drops to 33 3 percent while palmitic acid rises to 34.4 percent In biopsy 3, oleic acid level increases to 37 8 percent while palmitic acid drops to 26.3 percent. This complementarity is seen for each successive biopsy throughout the experimental period The levels of linoleic, stearic, palmitoleic, and myristic acid constitute relatively constant and minor components of the total fatty acids in this bird Stearic acid level rose to 18 7 percent in biopsy 3, from a 7 9 percent level in biopsy 1. Stearic acid levels in the remaining five biopsies remained below 13.0 percent Linoleic acid levels were highest in biopsies 1 and 2, at 13-1 percent for both, and declined gradually to 4.1 percent in biopsy 8 Myristic and palmitoleic acid levels

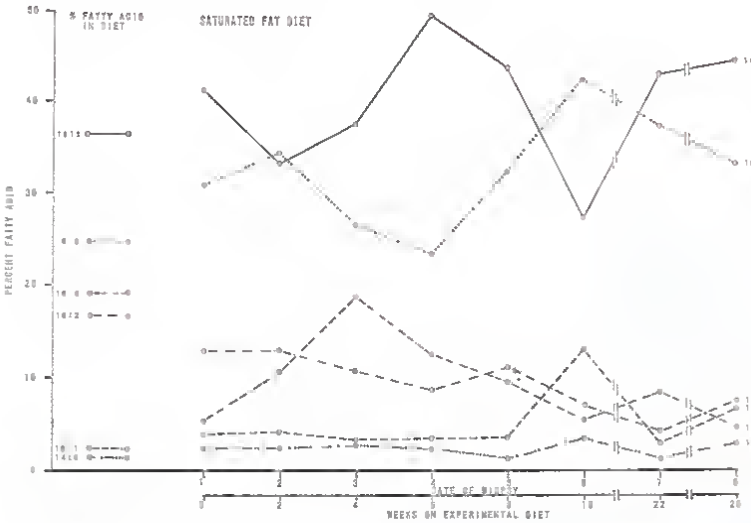

Figure 8. Effect of a saturated fat diet on the percent of fatty acids in an adipose tissue biopsy from the interfurcular fat pad of an Indigo Bunting.

did not rise above 4 percent in any tissue sample except in biopsies 6 and 8. In biopsy 6, palmitoleic acid rose to 13.6 percent and, after dropping to 3.1 percent in biopsy 7, it again rose to 6.7 percent in the last biopsy.

The percentage of fatty acids in the bird kept on the minimal fat diet is presented in Figure 10. Oleic and palmitic acids are, again, the major fatty acids constituting over 69 percent of the total fatty acids of every biopsy.

The complementary relationship described in the birds on the unsaturated and saturated fat diet is absent here, in the bird on the minimal fat diet. The levels of oleic and palmitic acid remained quite constant for all biopsies. Percentages of the remaining fatty acids, *i.e.*, linoleic, stearic, palmitoleic, and myristic, again constitute relatively constant components of the total fatty acids. Levels of these fatty acids are slightly lower in this bird than in the birds on the unsaturated and saturated fat diet. Except for the linoleic acid level in biopsy 1, which is 15.5 percent, the percent of linoleic acid is less than 8 percent of the total fatty acids for biopsies 2-7. Stearic, palmitoleic, and myristic acids, separately, do not exceed 8 percent of the total fatty acids for any biopsy.

The percentage of fatty acids in the bird kept on the control fat diet is presented in Figure 11. In all biopsies except biopsy 5, oleic and palmitic acids are the major fatty acids constituting over 65 percent of the total fatty acids of every biopsy. In biopsy 5, linoleic acid, at 35.9 percent, was the major fatty acid, with oleic acid representing

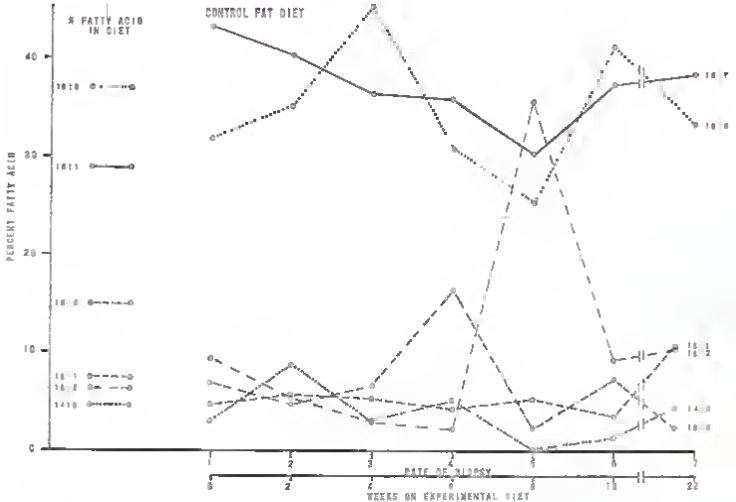

Figure 10. Effect of a minimal fat diet on the percent of fatty acids in an adipose tissue biopsy from the interfurcular fat pad of an Indigo Bunting.

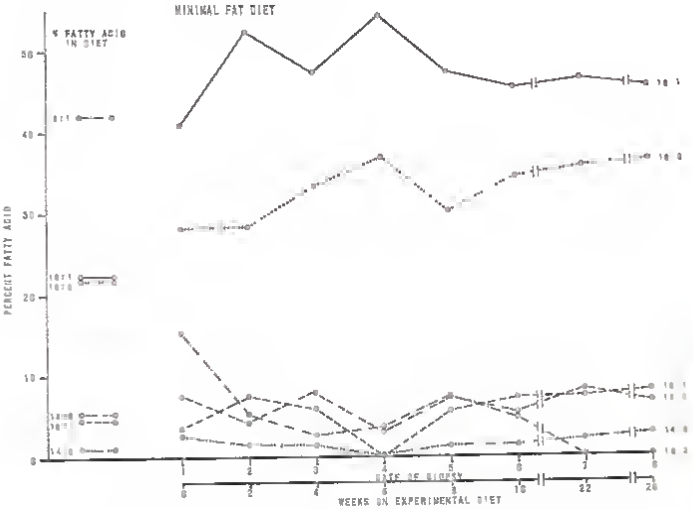

Figure 11. Effect of the control fat diet on the percent of fatty acids in an adipose tissue biopsy from the interfurcular fat pad of an Indigo Bunting.

25.7 percent of the total fatty acids. In all other biopsies linoleic acid occurred at levels of 2.5 percent to 10.9 percent. Although oleic acid levels do not vary greatly between biopsies 1 and 7, palmitic acid levels do, ranging between 25.6 percent and 45.6 percent. The complementary relationship described in the birds on the unsaturated and saturated fat diet is absent here, as in the case of the bird on the minimal fat diet. Stearic, palmitoleic, and myristic acids again constitute relatively constant components of the total fatty acids. Stearic acid in biopsy 4 increased to 16.7 percent, but in all other biopsies this fatty acid constituted less than 8 percent of the total fatty acids.

In order to determine whether the effects of time alter the total saturated fatty acid levels, myristic acid, palmitic acid and stearic acid are added together for each biopsy. The sum of all saturated fatty acids from each biopsy can then be compared (Figure 12). Included in this figure is the sum of saturated fatty acids in each diet. No trends in the levels of saturated fatty acids are apparent throughout the five and one-half months of the experimental period in birds on any of the diets.

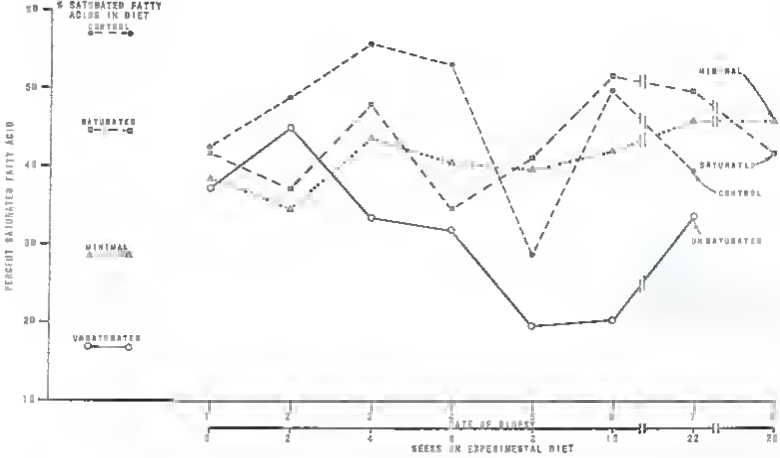

Figure 12. Effect of each diet on the sum of all saturated fatty acids, *i.e.*, myristic acid, palmitic acid and stearic acid found in adipose tissue biopsies from the interfurcular fat pad of Indigo Buntings.

DISCUSSION

Body Weights of Caged Indigo Buntings

In order to observe the possible influence dietary fatty acid composition might have on depot fat in the Indigo Bunting, it was essential that captive birds undergo some fat deposition. Although a quantification of depot fat in the interfurcular fat pad was not made, it was apparent that prior to starvation, at the time the first biopsy was taken on March 24, noticeable interfurcular fat pads were present in 27 out of 31 birds. Conversely, during the starvation period a conspicuous reduction in size of this fat pad was apparent. Helms and Drury (1960) found that interfurcular fat pad size in the Tree Sparrow (*Spizella arborea*) and the Slate-colored Junco (*Junco hyemalis*) correlated with diurnal weight changes. From data in Figure 1, the statistical analysis presented in the results, and the observed increase in fat pad size following initiation of the new diet, the increase in body weight experienced between March 31 and April 12 may therefore be accepted as a function of the increase in fat deposition.

Although a significant diminution in body weight occurred during the starvation period as might be expected, no statistically significant changes in mean body weights were discerned seasonally. There are several possible explanations for these results. The great variability among the body weights is apparent in the ranges and standard deviations presented

for each mean in Figure 1. The mean weights indicated seasonal trends, *e.g.*, the increased weights recorded during the winter of 1968 and the fall of 1969. A second possible explanation relates to the presence of an *ad libitum* food supply. Although seasonal increases in body weight of captive birds have been suggested by Helms (1963a), he has also indicated that these weight changes may be an "artifact of abundant food" (1963b). In the present study seasonal increases in the Indigo Bunting's weight may be affected by the same artifact. Decreases in body weight cannot be explained in the presence of "abundant food." The data which Helms (1963a) presents suggesting seasonal weight changes in captive White-throated Sparrows also emphasizes one other point. Three of the four sparrows for which he showed data were kept "under natural conditions." Fat deposition and reduction in these three birds is more evident than in the fourth which was kept at a constant room temperature (ca 25°C). The fact that the present study was also done at room temperature indicates a third possible reason why the captive birds were not capable of showing clear seasonal fat depositions. Thus, captivity and the factors associated with it (*ad libitum* food schedules, constant temperature, etc.) may possibly have interfered with fat deposition in caged Indigo Buntings.

Analysis of Fatty Acid Compositions

Analysis of the first biopsy taken as fat deposits began to accumulate indicated that the fatty acid composition of 27 pre-experimental Indigo Buntings was very similar to adipose tissue taken from the feral

migratory Wood Thrush in summer (Rosen, 1966), Coturnix Quail (McMullen, 1968), and several mammals including dog (Campbell, 1969), mouse (McMullen, 1968) and man (Kingsbury and Morgan, 1964). For example, oleic acid represents about 39 percent of the total fatty acids present in the dog adipose tissue, 44 percent in man, 42 percent in mice, 38 to 49 percent in the Wood Thrush, 36 percent in the Coturnix Quail, and 41 percent in the pre-diet Indigo Bunting. The profile of fatty acid concentrations in the pre-experimental buntings is noticeably different from the spring Junco (Bower and Helms, 1968) and five species of birds studied by Walker (1964). For example, oleic acid represents about 29 percent of the total fatty acids present in the Junco and 20 percent to 29 percent for the five species observed by Walker. Also, linoleic acid in the pre-diet bunting represented about 14 percent of the total fatty acids present, whereas it represented 39 percent of the fatty acids in the Junco. Since Bower and Helm's observations were based on entire bird extracts, they are not directly comparable to the present analyses which were based on adipose tissue extracts. In Walker's five species, oleic acid, at 20 percent to 29 percent, was the major fatty acid as in the Indigo Bunting, however, these levels were much lower than the 41 percent amount observed in the bunting. The similarities of fatty acid compositions between birds and mammals may be a function of the birds' being either caged or in a non-migratory state. These similarities may also be due to a lack of any adaptive importance to specifying fatty acid composition. The dissimilarities among the birds mentioned above may be a function of caged vs. feral birds, time of year of the analysis, the degree of fatness,

metabolic and dietary factors as well as species specificity.

Six major fatty acids (14:0, 16:0, 16:1, 18:0, 18:1, and 18:2) constitute nearly 100 percent of the fatty acids found in the depot fat of the Indigo Bunting. The percent of each of these fatty acids in the diet can be compared with the same fatty acid in the bird's adipose tissue after exposure to the diet. Figures 2 through 7 present these comparisons. When the fatty acid content in the diet and the first biopsy shows similar percentages of the total fatty acids, no distinct conclusions about the effect of diet on the bird's fat can be made. When the fatty acid percentage in the diet is significantly different from the level of the same fatty acid in the first biopsy, the effects of the diet on the tissue fatty acid level can be assessed. If the dietary level differs at least two standard deviations from the fatty acid level in the first biopsy, the inference of direct dietary influence on fat composition seems merited. For example, in Figure 2 each graph represents the change with respect to time of myristic acid (14:0) in a bird given one of four specific diets. In the case of this acid, it is difficult to determine whether or not the percent composition of the dietary myristic acid has a major effect on myristic acid level in the depot fat. This is because the percent composition of this fatty acid in all diets is within the $\pm$ 2 standard deviation range of variability of the first biopsy, and the percent composition of myristic acid in the depot fat rarely exceeds this range.

In the case of the palmitic acid (Figure 3), variability from biopsy to biopsy again is apparent. This is especially true in the birds main-

tained on the control and high saturated fat diets. In both of these cases it is, therefore, difficult to determine how much effect the dietary level of palmitic acid has on the depot level. However, in the bird on the high unsaturated fat diet, and the bird on the minimal fat diet, certain trends can be discerned. In the former situation, a lowering in the tissue level of palmitic acid (from 27 percent to 15 percent) to approach the percent composition of the diet (13 percent) was apparent. The level in the last biopsy reversed this trend, however. In the bird on the minimal fat diet, a gradual increase in percent composition of palmitic acid was seen rising from 28 percent to 36 percent, although the level of the palmitic acid in the diet was 22 percent. Although this change is within the $\pm$ 2 standard deviation range, it is interesting to note that the direction of change of the palmitic acid level in this bird was away from the palmitic acid level in the diet.

Comparing the effects of diet on stearic acid levels, with respect to time, certain changes are evident (Figure 4). As in the effect of the high unsaturated diet on palmitic acid level, the stearic acid level in the high unsaturated fat diet tends to approach the level of stearic acid in the diet. Stearic acid level in the high saturated fat diet was clearly greater than the $\pm$ 2 standard deviation range of variability for the stearic acid level in the bird on this diet (3 percent to 8 percent). After four weeks (30 April), the level of stearic acid in the depot increased and approached the level of this saturated fat in the diet (18.5 percent) but keeping the bird on the diet resulted in a lowered percentage level in the last two samples taken after 22 and 26 weeks, similar

to that in the first biopsy (6 percent). The bird on the minimal fat diet showed the least variability of stearic acid with the depot levels fluctuating around the dietary level.

The effect of diet on palmitoleic acid (16:1) is less pronounced (Figure 5) The level of this fatty acid is below the ± 2 standard deviation range of variability in the case of the bird on the high unsaturated fat diet. Only in the bird on this diet was the possibility of a dietary influence indicated but this effect is variable and took over ten weeks to appear. The range of variability includes palmitoleic acid levels in the other three diets, but the tissue fatty acid levels were also within the range of variability for almost all points. Therefore, no conclusions regarding dietary influence on this fatty acid can be made from the data available in this study

The direct influence of a dietary fatty acid on a depot fatty acid was more readily seen in the changes in percent composition of oleic acid (18:0) in the bird on the high unsaturated fat diet (Figure 6). The range of variability of this fatty acid in pre-experimental birds was 32.1 percent to 49.3 percent. The level of this fatty acid in the unsaturated diet was 20 percent. By the fourth biopsy, eight weeks after the bird had been placed on the unsaturated fat diet, the percent concentration of oleic acid in the depot fat was approximately 20 percent The level of oleic acid in this bird remained at about 20 percent through the remainder of the 22-week experimental period.

This direct influence which a dietary fatty acid imposed on its depot complement was clearly contrasted with the effect the oleic acid

in the minimal fat diet had on the level of oleic acid in the depot fat No apparent effect is seen when comparing levels of oleic acid in the depot fat of the bird maintained on this minimal fat diet In Figure 6 the level of dietary oleic acid is well below the level of depot oleic acid in the last four biopsies from this bird. Thus, the dietary fatty acid in this case does not appear to influence directly the concentration of oleic acid in adipose tissue.

The effects of the other two diets on this fatty acid are not as easily interpreted. In the bird kept on the control diet, the oleic acid composition in the depot fat seems to be undergoing a change in concentration (from 43 percent to 30 percent) which approaches the dietary level (29 percent) However, at the tenth week the depot oleic acid level rose to 38 percent so that the overall trend after 22 weeks is not clear. The values of the oleic acid levels in the high saturated fat diet are in the range of variability and no effect is easily seen here.

Direct and indirect effects of a dietary fatty acid were also seen in the case of linoleic acid (18:2)(Figure 7). In the bird kept on the high unsaturated fat diet, the percent concentration of linoleic acid in the depot fat began to approach the concentration of the dietary linoleic acid by the sixth week, [illegible], from 14 percent to 47 percent, and continued to increase to over 60 percent by the tenth week The dietary level of linoleic acid was 63 percent. Although the seventh biopsy point indicates a lowering of linoleic acid to 43 percent, the direct influence of the dietary linoleic acid is clear since the dietary level of the acid is far above the range of variability. The linoleic acid levels in the bird on

the minimal diet showed a different response. In this bird the level of linoleic acid dropped from 15 percent to zero by 22 weeks on the diet while the dietary level was over 42 percent. As in the case of the oleic acid, the dietary level was clearly out of the range of the limits of variability for linoleic acid but the depot fatty acid did not reach levels equivalent to the linoleic acid levels in this diet. In the control and high saturated fat diet, variability was generally small and was close to the percent concentration of the dietary linoleic acid, making it difficult to discern dietary influence upon depot fatty acids

The most apparent direct influence suggested by this study was the variation in depot fatty acids in biopsies taken from the bird on the high unsaturated fatty acid diet With the exception of myristic acid, tissue levels of fatty acids in this bird seem to be influenced in varying degrees by the percentage in the diet of the same fatty acids. The acids in the bird's depot fat were changed from their initial levels to approach, either by increasing or decreasing, their respective levels to that of the diet These results support the reports by Cruikshank (1934), Mickelberry *et al* (1966) and others who reported influences on chicken depot fat by using diets containing high percentages (10 percent or greater) of unsaturated fatty acids The most significant response indicating an inability of the dietary complement to influence directly the depot fatty acids is seen in the minimal fat diet, particularly in the cases of oleic and linoleic acid and possibly with reference to palmitic acid, as well.

The lack of a clear-cut relationship between dietary fatty acids and depot fat as seen in the bird on the minimal fat diet is not without precedent. Edwards (1962) found that chickens raised for 25 days on

diets containing less than 8 percent triolein, safflower oil or menhaden oil did not alter the relative proportions of oleic acid and palmitic acid in depot fat samples. Such results are consistent with those found in the present study, i.e., oleic acid, linoleic acid and, to a lesser degree, palmitic acid levels in the bird on the minimal fat diet were not influenced by these fatty acids in the diets. West and Meng's report (1968a) of dietary effects on depot fatty acids in the Redpoll tend to agree with those of Edwards. They found that dietary fatty acids did not influence depot fatty acids in birds on three different diets. Since the fatty acids of the minimal fat diet in the present study did not seem to influence the depot fat of the bird kept on that diet, it is possible that the percentage of fat in the diets West and Meng gave the Redpolls was not large enough for the fatty acids in the diet to influence the tissue. It should also be pointed out that West and Meng's study was terminated after six weeks whereas in the present study six weeks were required to see the first signs of any dietary influence of fatty acids. All of these results suggest that although fat deposition may occur in a non-migratory or migratory bird, dietary fat quantity, as well as quality, might determine the influence of the dietary fatty acids on specific fatty acid levels in the depot fat.

When the data were regrouped by bird rather than by fatty acid (Figures 8 through 11), an interesting complementary relationship was seen between oleic acid (18:1) and palmitic acid (16:0) levels. This complementarity was noted only in the birds on the high unsaturated and

high saturated fat diets. In the case of the saturated fat diet, it is possible that because these two components made up the major portion of the total fatty acids present, the observed effect is purely a numerical complementarity. That is, a change in the level of one fatty acid which constitutes 30 to 40 percent of the total fatty acids, is capable of altering the level of another fatty acid which represents a similar high percent of the total fatty acids. Gellhorn *et al.* (1962) indicated that levels of palmitic, palmitoleic, and oleic acids decreased in total lipids in rats kept on a 60 percent linoleic acid diet, as the linoleic acid level increased. This argument doesn't seem acceptable in the present case, however, for in the bird on the high unsaturated fat diet (Figure 8) the same complementary relationship between palmitic and oleic acids was present even though linoleic acid was the major fatty acid in four of the seven biopsies analyzed. What might be suggested from these complementary relationships is that an effective alteration (either metabolically or dietarily controlled) in the depot levels of one of these two fatty acids could induce a complementary change in the other, if the diet contains high levels (over 11 percent) of fat. This complementarity was not detected in the bird on the minimal fat diet, meaning that this bird was maintaining physiological levels of fatty acids regardless of the dietary fatty acid components. This latter point is supported by the earlier observation that the fatty acid levels in the depot fat do not seem to be influenced directly by the fatty acid concentration of the minimal fat diet. Swoer and Helms' results (1968) do

not indicate a palmitic-oleic acid reciprocal relationship but they do report that palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) "were the major acid components varying seasonally." Reiser and Raju (1964) showed that the fatty acid levels in the diet may alter endogenous syntheses of fatty acids. Although this is not a specific effect where a particular fatty acid in the diet inhibits endogenous synthesis of the same fatty acid (Carroll, 1965), it may result in the type of palmitic acid-oleic acid relationship discussed here.

Figures 8 to 11 also illustrate the possible influence time may have on each fatty acid concentration in a given bird on its diet. The question arises as to whether there are changes in percent composition of the fatty acids of adipose tissue as fat deposition increases. Nakamura (1962, 1969), McGreal and Farner (1956), and Rosen (1966) all reporting on migratory birds and West and Meng's (1968a) study on a non-migratory bird discussed the influence seasonal changes might have on adipose fatty acid composition relative to migration and breeding. Nakamura, West and Meng, and Rosen have indicated possible seasonal changes in depot fat composition as the season progressed, whereas McGreal and Farner found no significant changes. Because the present study was done on captive birds, it is impossible to make any definite statements regarding seasonal alterations in the fatty acid composition of depot fat in feral birds. However, by summing all saturated fatty acids (Figure 12), any changes with respect to time in each bird may be more easily seen than in Figures 8 to 11 where individual fatty acids are considered

separately. From the data in Figure 12, it can be seen that, with respect to time, no variability in saturated fatty acids is apparent which cannot be accounted for by dietary influence as in the case of the bird on high unsaturated fat diet or by constant (as opposed to varying) physiologically maintained levels as in the case of the bird on the minimal fat diet.

The possibility has been discussed of physiologically controlled, endogenously synthesized, depot fatty acids as suggested in the fatty acid levels of the bird on the minimal fat diet. Regarding this possibility of metabolic control, the biochemical relationship between these metabolites should be discussed. It has been shown that the precursor of linoleic and linolenic acid in plant leaf homogenates is oleic acid (James, 1962), in its active form oleyl - SCoA. Similar studies on leaf chloroplasts (Harris, Hames and Harris, 1967, Nagai and Black, 1966) indicated that oleic acid is derived from stearate. This latter reaction occurs in rat and chicken liver preparations as well (Johnson, *et al*, 1967).

The desaturation of oleic acid to linoleic and of linolenic acid has not been observed in animal tissue, however, Johnson, *et al* (1967) discussed the effect of the cyclopropenoid sterculic acid on inhibiting the desaturation reaction from stearate to oleate. They pointed out that chickens raised on cottonseed oil, which contains high concentrations of this inhibitor, show a concomitant hardening of depot fat as the oleic levels decrease and stearic levels increase. In the present study, there did not appear to be any noticeable change in the texture of the biopsied

fat from the Indigo Bunting's depot fat throughout the 6-month period observed, with one exception. Biopsies from the birds on the high unsaturated fat diet did become noticeably softer. A second important point to be gained from Johnson, *et al.*, relates to the fact that the fatty acid compositions did not vary directly with the fatty acid levels in the diet, but with the presence of a metabolic inhibitor in the diet.

Thus, it seems that there are five possible influences between dietary fatty acids and the fatty acid composition of depot fat:

1. Depot fat composition is strictly a function of dietary composition. This has been indicated in most studies of non-migratory domestic and game birds and the short-distance migrant, Slate-colored Junco. This method is supported in part by the present study and seems to occur when the concentration of fat in the diet is above some minimal level.
2. Dietary fat does not affect the fatty acid composition of the depot fat. This alternative was supported by Edwards in his study on chickens and West and Meng in their study on the Redpoll. The present work on migratory Indigo Bunting supports this possibility only when the dietary supplement of fat is around 1 percent of the diet.
3. Changes in metabolic states, as migratory birds enter and leave different periods of their annual cycle, may alter fatty acid levels in adipose tissue. The Great Reed Warbler and the Wood Thrush showed seasonal changes in unsaturation and saponi-

fication number (Nakamura, 1962) and 18:2/16:0 ratio (Rosen, 1966) which both authors attributed to metabolic changes; however, it cannot be ascertained at present whether or not the birds in question altered their diets. There is no evidence in this study to support this alternative.

4. Metabolites in the birds' diet could indirectly influence fatty acid composition. Johnson, *et al.* indicated this possibility in discussing the effect of the natural inhibitor, sterculic acid.

5. Various combinations of the above. The possible compelmentarity between palmitic and oleic acid in the present study may be an example of this. If either acid is affected by the amount of that fatty acid in the diet a complementary change may be metabolically induced, also.

Since the present study seems to support points 1, 2, and 5, we may tentatively conclude that dietary fatty acids can influence depot fatty acid compositions if the percent of dietary fatty acid is above some as yet undetermined level. In the Indigo Bunting this was seen when the percentage of fat in the diet was about 5 percent, but was not apparent at 1 percent. If the fat percent is high enough to influence fatty acid tissue levels, it is possible that altered levels in one fatty acid may influence other fatty acid levels present through intermediary metabolism in the liver or adipose tissue. If the amount of dietary fat is below some minimal level, the depot fat may establish a physiological level without regard to the percent concentration of the fatty acids in the diet.

In order to clarify better the role dietary fatty acids have in influencing depot fatty acids, studies on the fatty acid composition and the percentage of fat in the seasonal diets of feral migrants are essential. In laboratory controlled studies, methods for developing larger sample sizes than those which were used in the present study are equally as essential. The amount of time required for dietary fatty acids to effect changes in the depot fats should also be considered The results of the present study indicate that this interval may be six to ten weeks in length in the Indigo Bunting. Therefore, extensive observations and analyses of premigratory food and depot fats will be necessary before the effects of exogenous influences in depot fatty acids can be separated from endogenous physiologically controlled fatty acid levels in migratory birds.

SUMMARY

Fatty acid analyses of biopsies from the interfurcular fat pad of captive Indigo Buntings were presented. Mean predictory percentages of each fatty acid for the 28 birds tested were: myristic acid (14:0), 2.83 percent, palmitic acid (16:0), 29.36 percent, stearic acid (18:0), 6.08 percent, palmitoleic acid (16:1), 6.10 percent, oleic acid (18:1), 40.66 percent, and linoleic acid (18:2), 14.43 percent.

The fatty acid compositions of depot fat from four birds, each one kept on a different diet of known fat percentage and fatty acid composition were presented. When each fatty acid in the diet was compared with the same fatty acid in the fat biopsy, the following observations could be made: myristic acid levels in the diets and the depot fats were too similar to determine any possible influence dietary myristic acid might have on depot levels.

Palmitic acid (16:0) and palmitoleic acid (16:1) levels in the bird on the unsaturated diet dropped with respect to time approaching their dietary levels. In the bird on the minimal fat diet, the trend in palmitic acid levels was away from dietary levels whereas this trend was not evident in palmitoleic acid levels. Stearic acid levels tended to approach dietary levels in the bird on the unsaturated fat diet. The clearest results indicating dietary influence on depot fatty acids is seen in changing oleic acid and linoleic acid levels in the bird on the

unsaturated fat diet The clearest results which indicate a lack of influence of dietary fatty acids on depot levels is seen in oleic acid and linoleic acid levels in the bird on the minimal fat diet In the birds kept on the unsaturated fat and saturated fat diets, a complementary relationship seemed to exist between oleic and palmitic acid levels

Dietary influences of depot fatty acids may occur when percentage of fat in the diet is above some minimal level. Below this level, the percent of fatty acids in depot fat may be under some endogenous control.

LITERATURE CITED

Barnett, L.B. 1970. Seasonal changes in temperature acclimatization of the house sparrow, *Passer domesticus*. Comp. Biochem. Physiol., 33(3): 559-578.

Bower, E.B. and C.W. Helms. 1968. Seasonal variation in fatty acids of the Slate-colored Junco (*Junco hyemalis*). Physiol. Zool., 41: 157-168.

Campbell, R.G. and S. Hashim. 1969. Deposition in adipose tissue and transport of odd-numbered fatty acids. Amer. J. Physiol., 217(6): 1614-1618.

Carroll, K.K. 1965. Dietary fat and the fatty acid composition of tissue lipids. J. Amer. Oil Chem. Soc., 42: 516-528.

Cruickshank, E.M. 1934. Studies in fat metabolism in the fowl. I. The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amounts of dietary fat. Biochem. J., 28: 965-977.

Dolnik, V.R. and T.I. Blyumental. 1967. Autumnal premigratory and migratory periods in the Chaffinch *Fringilla coelebs coelebs*, and some other temperate zone passerine birds. Condor, 69(5): 435-468.

Edwards, H.M., Jr., J.E. Marion and J.C. Diggers. 1962. Studies on fat and fatty acid requirements of poultry. Proceedings 12th World Poultry Congress, 182-186.

Feigenbaum, A.S. and H. Fisher. 1959. The influence of dietary fat on the incorporation of fatty acids into body and egg fat in the hen. Arch. of Biochem. and Biophys., 79: 302-306.

Folch, J.I., M. Ascoli, M. Lees, J.A. Meath, and F.N. LeBaron. 1951. Preparation of lipid extracts from brain tissue. J. Biol. Chem., 191: 833-841.

Fry, C.H. 1967. Lipid levels in an intratropical migrant. Ibis, 109: 118-120.

Gellhorn, A., W. Benjamin, and M. Wagner. 1962. The *in vitro* incorporation of acetate-1-C^{14} into individual fatty acids of adipose tissue from young and old rats. J. Lipid Res., 3(3): 314-319.

Gunstone, F.D. 1958. An introduction to the chemistry of fats and fatty acids. John Wiley and Sons, Inc., New York.

Harris, R.V., A.T. James and P. Harris. 1957. In "The Biochemistry of Chloroplasts," Volume II, p. 241, edited by Goodwin, T.W., Academic Press, Inc., New York.

Helms, C.W. 1963. The annual cycle and *migrants* in birds. Proceedings XIII International Ornithological Congress, 925-939.

Helms, C.W., and W.H. Drury, Jr. 1960. Winter and migratory weight and fat field studies on some North American buntings. Bird-banding, 31(1):1-40.

Hilditch, T.P. 1964. The chemical constitution of natural fats. 4th Ed., John Wiley and Sons, New York.

Horning, E.C., E.M. Ahrens, Jr., S.R. Lipsky, F.H. Mattson, J.F. Mead, D.A. Turner, and W.H. Goldwater. 1964. Quantitative analysis and fatty acids by gal-liquid chromatography. J. Lipid Res., 6:20-27.

Isaaks, R.E., R.F. Davey, T.M. Ferguson, R. Reiser and J.R. Couch. 1964. Studies on avian fat composition. I. Effect of dietary fat on the fatty acids of the tryglyceride of phospholipid fractions of the blood plasma and adipose tissue lipids of the laying hen. Poultry Sci., 43:105-120.

James, A.T. 1962. The biosynthesis of unsaturated fatty acids in isolated plant leaves. Biochem. Biophys. Acta, 57:167.

James, A.T. 1967. The biosynthesis of unsaturated fatty acids in photosynthetic systems in lower and higher plants. In Cellular Compartmentalization and Control of Fatty Acid Metabolism, Gran, F.C., Ed., Academic Press, New York.

Johnson, A.R., J. Pearson, F.S. Shenstone and A.C. Fogerty. 1967. Inhibition of the desaturation of stearic to oleic acid by cyclopropene fatty acids. Nature, London, 214:1244.

Johnston, D.W. 1965a. Ecology of the Indigo Bunting in Florida. Quart. J. Fla. Acad. Sci., 28(2):200-211.

Johnston, D.W. 1965b. An effective method for trapping territorial male Indigo Buntings. Bird-banding, 36(2):80-83.

Kingsbury, K.J., and D.M. Morgan. 1963. The fatty acid composition of breast, thigh and skin tissues of chicken broilers as influenced by dietary fats. Poultry Sci., 42:1202-1207.

McGreal, R.D , and S.D Farner 1956 Premigratory fat deposition in the Ganhel White-crowned Sparrow: some morphologic and chemical observations Northwest Sci , 30 12-23

McMullen, G F , S.C Smith, and P A Wright 1968. Tissue fatty acid composition in four diverse vertebrate species. Comp. Biochem Physiol , 26-211-221

Metcalf, L.D , and A.A Schmitz 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Analyt. Chem. 33: 363-364.

Mickelberry, W.C., M.C Rogler, and W.J Stademan 1966 The influence of dietary fat and environmental temperature upon chick growth and carcass composition Poultry Sci , 45 313-321

Morrison, D.E 1967 Multivariate Methods. McGraw-Hill, New York.

Moss, R and Lough, A K 1968. Fatty acid composition of depot fats in some game birds, Tetraonidae Comp Biochem Physiol , 25,559-562.

Nagai, J., and K. Block 1966. Enzymatic desaturation of stearyl Acyl carrying protein. J. Biol. Chem., 241-1925.

Nakamura, T. 1962 The change of body lipid amount and some chemical observations in migratory Eastern Great Reed-Warbler in the breeding ground. Miscellaneous reports of the Yamanashi's Institute for Ornithology and Zoology, 3(3) 185-188.

Nakamura, T 1963. Changes of lipids in Eastern Great Reed-Warbler Chemical Observations, Memoirs of the faculty of Liberal Arts and Education, Yamanashi University, Japan, 14 141-149

Nakamura, T 1969. Physiological and ecological studies on bird migration with special reference to body lipids, Tori, 19 87-92.

Odum, E P. 1960. Premigratory hyperphagia in birds Amer. J. Clin Nutr., 8 621-627.

Odum, E P., and J C. Major 1956. The effect of diet on photoperiod-Induced lipid deposition in the White-throated Sparrow, Condor, 58 222-228

Odum, E P., and J.D Perkinson 1951. Relation of lipid metabolism to migration in birds. Seasonal variation in body lipids of the migratory White-throated Sparrow, Physiol Zool., 24 216-230

Reiser, R., and P K Raju 1964 The inhibition of saturated fatty acid dehydrogenation by dietary fat containing sterculic and malvalic acid, Biochem Biophys. Res Commun , 17(1) 8-11

Rosen, E. 1966. A seasonal analysis of fatty acids in the Wood Thrush (*Hylocichla mustelina*). Rutgers, The State University, University Microfilms, Inc., Ann Arbor, Michigan.

Sell, J.L., S.H. Choo, and P.A. Kendra. 1968. Fatty acid composition of egg-yolk and adipose tissue as influenced by dietary fat and strain of hen. Poultry Sci., 47(4): 1296-1302.

Shorland, F.B. 1950. Effect of the dietary fat on the composition of the depot fats of animals. Nature, 165:766.

Tanhuanpaa, E., and E. Pulliainin. 1969. Major fatty acid composition of some organ fats in the Willow Grouse, *Lagopus lagopus* and the Rock Ptarmigan, *Lagopus mutus*. Ann. Acad. Sci. Fenn., Series A, 4:1-14.

Walker, A.T. 1964. Major fatty acids in migratory bird fat. Physiol. Zool., 37:57-64.

Ward, P. 1965. Feeding ecology of the Black-faced Dioch, *Quelea quelea*, in Nigeria. Ibis, 107:173-214.

West, G.C., and M.S. Meng. 1968a. The effect of diet and captivity on the fatty acid composition of Redpoll *Acanthea flammea* depot fats. Comp. Biochem. Physiol., 26:535-540.

West, G.C., and M.S. Meng. 1968b. Seasonal changes in body weight and fat and the relation of fatty acid composition to diet in the Willow Ptarmigan, Wilson Bull., 80(4): 426-441.

White, A., P. Handler, and E. Smith. 1968. Principles of Biochemistry. 4th Ed., McGraw-Hill Book Company, New York.

Yarbrough, C.G., and D.W. Johnston. 1965. Lipid deposition in wintering and premigratory Myrtle Warblers. Wilson Bull., 77(2): 175-191.

BIOGRAPHICAL SKETCH

David L[illegible] born June 17, 1939, in the Bronx, New York. He graduated from Jam[illegible] Monroe High School in the Bronx, June, 1955. In June, 1959, he received the degree of Bachelor of Arts with a major in chemistry from Western Reserve University. He worked as a high school biology teacher in New York City and then as a laboratory technician at the University of Miami's Institute of Marine Sciences. In September, 1964, he enrolled in the Graduate School of the University of Florida. In April, 1966, he received the degree of Master of Science in Teaching from the University of Florida. He worked as a graduate assistant in the Department of Biological Sciences from September, 1966, until June, 1967. From July, 1967, until September, 1969, he was a graduate assistant in the Department of Ophthalmology. From September, 1969, to the present he has been a research associate in the Department of Ophthalmology. Upon satisfactory completion of his graduate training he will begin a two-year Post-doctoral Fellowship awarded to him by the National Eye Institute in the Department of Anatomy of the University of New Mexico School of Medicine.

David Landau is married to Marcia Landau and has one child, Michelle, three years old. He is the past president of the Sigma Chapter of Phi Sigma and is a member of the American Institute of Biological Sciences, the American Association for the Advancement of Science, the Audubon Society and the Association for Research in Ophthalmology.

This dissertation [illegible] under the direction [illegible] the chair[illegible] of the candidate's supervi[illegible]ittee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology.

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